

CLAIMS

1. Nucleic acid comprising at least one segment of the gene encoding a functional portion or the gene-regulating region of the alpha 2 subunit of the Na,K pump (ATPase, ATP1A2) for use in the diagnosis of pathologies associated with migraine or with alternating hemiplegia of the childhood.
- 5 2. Nucleic acid comprising at least one segment of the gene encoding a functional portion or the gene-regulating region of the alpha 2 subunit of the Na,K pump (ATPase, ATP1A2) for use in genetic therapy for pathologies associated with migraine or with alternating hemiplegia of the childhood.
- 10 3. Method to detect in an individual at least one mutation in the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2) located on chromosome 1, associated with migraine disorders, which comprises the steps of:
 - collecting a sample containing a sufficient quantity of the individual's DNA or that is reproducible in culture;
 - isolating of the DNA from the sample;
 - exponential amplifying the DNA using as an oligonucleotide pair for the amplification reaction at least two oligonucleotides that are able to amplify at least one segment of the gene encoding the alpha 2 subunit of the Na,K
 - 15 human pump (ATPase, ATP1A2) or a segment of the region regulating it;
 - detecting in at least one amplified segment any mutations compared with a healthy control.
- 20 4. Method according to claim 3 in which the oligonucleotide pairs are:

17	AGTCCCTCTGACCTCCCTGAT	CCACTGTGCCATCACGATT	
25	19	CTTCTGCTTCCTGCTCTGACC	ACACATGTGCGCTGTGTTAC.
5. Method according to claim 3 in which the DNA exponential amplification phase is performed using oligonucleotide pairs that are able to amplify the entire encoding portion of the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2).
- 30 6. Method according to claim 5 in which the DNA exponential amplification phase to amplify the entire portion encoding the gene for the alpha 2 subunit

of the Na,K human pump (ATPase, ATP1A2) comprises the use of at least one of the following oligonucleotide pairs:

1	TGTTGCTTGGCTTCTCTGT	CTCCCTCACCCCTAGACTGC
2+3	CCCCTCTCTTCCCTGACTCT	GCCTCTTTGTTCCCTCCCTA
5	4 ATGGTGACTGGCTGGGTTG	CAGGGTTGGAGGACAGTCAC
	5 AGCTGCCCTTAGGGTTG	ACCTTACAGCCTAGCCCAGAG
	6 GAGACCAGCAGGAGAAGAAGG	AGACTCAACTGCTTGCTCTGG
	7 TACAAGTGGCTCTGCCAGTCT	AGCCCTTCATCCTGACTATGG
	8 CAGGAAATAGGATGGGACTGC	GTAGTGAGACCCCTCCCTGGT
10	9 ATCTCCGGCTTCAGCCTTAAC	TAATCCTATCCACCCCCCTCG
	10+11 CTCCTGGTCCCCCTCAT	TCCCTCTCTCTTCCCTGTCC
	12 GCGCTACCAAGACAAGTATGG	CTTGGGAATCCCCCTCTGAG
	13 GAAGCCACTCTGCGGATCT	ACTGCAGCTCCTGAACTCTG
	14 GGAGGGGGATAAACCTTAAT	GACGTGTTGATTAGGGCACAG
15	15 AGGGGTAGCTGTCTCTGTC	GGTCCCTGCCTGTCATCTG
	16 AAGGGGTTTCGTCCTCAAGT	TCAGTATCCTGCAAACCATCC
	17 AGTCCCTCTGACCTCCCTGAT	CCACTGTGCCATCACGATT
	18 TCATCTCCTACGTCCCTCAA	AGCTGGAAAAGAACCCCTGT
	19 CTTCTGCTTCCTGCTCTGACC	ACACATGTGCGCTGTGTTAC
20	20 CCTCCGACACTCTCATCTGTC	CTGTGTGGTTGGTGAGTGT
	21 CTTCACCTGCCACCTCCTT	CCCCCGTATGACTACTCAGG
	22 CGCTTGAATGCTCCTTATG	GAGGGAGGAGCTGGTGGT
	23 GCCTCCTTTAACGCTCATGCT	GCCTCATTATCTCTCCCCAAA
	7. Method according to claim 3 in which the DNA exponential amplification phase is performed using oligonucleotide pairs that are able to amplify the regulating region of the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2).	
25		
	8. Method according to claim 7 in which the DNA exponential amplification phase to amplify the regulating region of the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2) comprises the use of the following oligonucleotide pairs:	
	1_Pr TTCCCCCTCACTCCATCTCTG	GACCCCTGCTCTTAGGGATA
	2_Pr GATTCAAGGACCACTCCATCC	GGGAACAGTCAGAGGACAGG.

9. Method according to the aforementioned claims in which the detection phase of at least one amplified segment with any mutations compared with a healthy control is performed using direct sequencing or an SSCP method (single strand conformation polymorphism) (17) DHPLC or DGGE

5 (denaturing gradient gel electrophoresis) (18).

10. Diagnostic kit for pathologies associated with migraine or with alternating hemiplegia of the childhood to carry out the method according to claims 3 through 9, that comprises:

- at least one pair of oligonucleotides for the exponential amplification

10 reaction of at least one segment of the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2), in which the aforesaid segment encodes a functional portion or a gene-regulating portion of the aforesaid subunit;

- a control DNA from a non affected individual.

- 15 11. Kit according to claim 10 in which the oligonucleotide pairs for the amplification reaction are able to amplify the entire encoding region of the gene encoding the alpha 2 subunit of the Na,K human pump (ATPase, ATP1A2).

12. Alpha 2 subunit protein of the Na,K human pump (ATPase, ATP1A2) or a 20 functional portion thereof for use in the diagnosis of pathologies associated with migraine or with alternating hemiplegia of the childhood.

13. Alpha 2 subunit protein of the Na,K human pump (ATPase, ATP1A2) or a functional portion thereof for use in the treatment of pathologies associated with migraine.

25 14. Method for the identification of an agonist or antagonist agent of the Na,K human pump (ATPase, ATP1A2) or a functional portion or a gene-regulating portion of the subunit, that comprises:

(i) transfection of a cell line with a gene for a mutant isoform of the Na,K human pump (ATPase, ATP1A2) resistant to ouabain;

30 (ii) appropriate exposure of the transfected cells to the agent;

(iii) measurement of the Na,K pump activity in relation to ion transport with labeled ions.

15. Method for the identification of an agonist or antagonist agent of the Na,K pump (ATPase, ATP1A2) or a functional portion, that comprises the phases:

5 (i) use of the agent to treat a transgenic animal that expresses a mutant isoform of the Na,K pump (ATPase, ATP1A2) or that is partially or completely deleted in the gene encoding the Na,K pump (ATPase, ATP1A2) or

(ii) use of the agent to treat eukaryotic or prokaryotic cell lines that express mutant or normal forms of the Na,K pump (ATPase, ATP1A2) by transient or

10 stable transfection or in physiological conditions.